EXTRACTIVE TBA METHOD: A COMPARATIVE STUDY

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SUMMARY

There are different methods to measure the lipid oxidation in muscle foods. The thiobarbituric acid (TBA) test is one of the most popular and commonly used methods for this detection. This test can be performed by different ways (extraction, distillation, HPLC) which have advantages and disadvantages that have been discussed by different authors. The advantage of the extraction method is that it is quick and does not need sophisticated equipment, which makes it useful to be used in the meat industry. The aim of this work was to study the influence of different conditions on TBA extraction method. Different extracting agents, different concentrations of these acids, different reaction times and sulfanilamide use were evaluated. The highest TBA number was found in all samples with a reaction time of 35 minutes in a thermostatically controlled water-bath at 100 °C, rather than 900 minutes at room temperature. Significant statistical differences between 35 and 900 minutes of MA-TBA reaction time, for the different acid concentrations, were not found. The lowest TBA number was found in all samples with the addition of sulfanilamide rather than without it. The 10 % TCA solution showed the best recovery percentages for "paté", which made it preferable to other studied extracting solutions.

INTRODUCTION

Lipids can become rancid as a consequence of oxidation, and this is one of the most important change during food storage and production, since it produces its deterioration or takes part in its flavour (Pearson, 1977; Erikson, 1982).

There are different methods to measure this lipid oxidation in muscle foods. The thiobarbituric acid (TBA) test is one of the most popular and commonly used methods for this detection (Gray, 1978; Kakuda, 1981; Melton, 1983; Raharjo, 1993). The first evidence of this method was study carried out by Kwon (1944) which described the red colour pigment formation between animal tissues and TBA reagent. Later, Bernheim (1947) and Patton (1951) described that this red pigment was formed by the condensation of one malonaldehyde (MA) molecule with two TBA molecules. MA, the principal substance that reacts with TBA reagent (Patton, 1951), is a secondary oxidation product of polyunsatured fatty acids, containing three or more double bonds (Dahle, 1962) and is a highly reactive dicarbonyl (Kakuda, 1981).

There are some ways in which the TBA test can be performed on muscle foods (Patton, 1951; Turner, 1954; Sinnhuber, 1958; Tarladgis, 1960 and 1964; Witte, 1970; Rhee, 1978; Pikul, 1983 and 1989). In addition, the authors propose different times and temperatures to incube the TBA-MA reaction: 15 hs at room temperature (Tarladgis, 1964; Witte, 1970; Pikul, 1983) or 30-35 minutes in a boiling water bath (Turner, 1954; Tarladgis, 1960; Kakuda, 1981; Pikul, 1983; Shahidi, 1985 and 1987), as well as different extracting solutions: tricloracetic acid (TCA) in aqueous solution (Shahidi, 1990), TCA in phosphoric acid solution (Turner, 1954; Witte, 1970) or HCl solution (Zipzer, 1962). On the other hand, several modifications to the test have been done: a) the addition of sulfanilamide in TBA test for cured meat samples (Zipzer, 1962); b) the incorporation of an antioxidant to prevent the autoxidation during the distillation step (Pikul, 1983); c) the use of a high performance liquid chromatography (HPLC) method to avoid the interference of colour impurities in the spectrophotometric method (Kakuda, 1981; Csallany, 1984). The TBA test is a useful method to measure the lipid oxidative rancidity, but it is necessary to adapt this test to the sample conditions, because quantitative and qualitative problems could exist (Marcuse, 1973). The advantages of the extraction method are its simplicity and ease of useability (Witte, 1970).

The general aim of this work was to study the TBA extractive method to measure lipid oxidation in "pate". The particular aims of this work were to study the influence of different: a) extracting solutions (types and concentrations), b) times and temperatures of reactive incubation, and c) the use of sulfanilamide for this condition and product.

MATERIALS AND METHODS

1) Reagents: *TCA solutions*: 10, 15 and 20 % of Tricloracetic acid (TCA)(Panreac - Montplet & Esteban S.A.) in distilled water. *PA solution*: 2M Phosphoric acid (Panreac - Montplet & Esteban S.A.). *TCA+PA solutions*: 10, 15 and 20 % of TCA in 2M Phosphoric ácid. *TMP*: 1,1,3,3-Tetramethoxypropane (Merck-Schuchardt); a 10E-3 M TMP stock solution was prepared. *MA solution*: an aliquot of 30 µl of the TMP stock solution diluted in 5 ml of distilled water. *SF solution*: 0.5 % sulfanilamide (E. Merck-Darmstadt) in 20 % HCl (Panreac- Montplet & Esteban S.A.). *TBA solution*: 0.02 M 2-thiobarbituric acid (E. Merck-Darmstadt) in distilled water. All chemicals used in this study were reagent-grade commercial products and were used without any further purification.

2)TMP standard curve: aliquots of 10 µl to 100 µl of the TMP stock solution were pipetted into assay tubes with a High Tech Lab Precision Pipette (Model VE-1000xr) and diluted to 5 ml with distilled water. Then, 5 ml of TBA solution was added to each tube, which were covered with Prafilm, mixed by invertion and placed in a thermostatically controlled water-bath for 35 minutes, at 100 °C, to allow the development of the color reaction (MA-TBA). The tubes were cooled down with tap water. The experiment was repeated by placing the tubes in the dark for 15 hours, at room temperature, to develop the color. In both cases the absorbance of each tube was measured by an HP 8451A Diode ARRAY spectrophotometer at 532 nm. Two standard curves were prepared and the results are plotted in figures 1 and 2.

3) Methodology: the "paté" was elaborated as described previously (Perez-Alvarez, 1993). Prior to taking the samples, the residual nitrite level was determined by the standard ISO/DIS 2918 method (M.A.P.A., 1985). Samples of 4 g (± 0,001 g) were introduced into centrifuge tubes. Then, for the sulfanilamide effect study 1 ml of SF solution or 1 ml of distilled water was incorporated. Subsequently, 10 ml of one extracting solution (10, 15 or 20 % of TCA or 10, 15 or 20 % TCA-PA) and for the MA percent recovery study 5 ml of MA solution or 5 ml of distilled water were added. The tubes were stirred for 5 minutes with a Heidolph vortex stirrer (model REAX 2000). At the end of this time, 5 ml of TBA solution was added to each tube, which were stirred again for 2 minutes. Each tube was centrifuged for 5 minutes, at 3500 rpm, in a Selecta Meditronic centrifuge, to separate the liquid phase. This phase was filtered through Whatman n° 1 filter paper collecting it into assay tubes. Later, the tubes were placed in a thermostatically controlled water-bath for 35 minutes at 100 °C, or 15 hours in the dark, at room temperature, to allow the development of the MA-TBA colour complex. In all experiments the absorbance, of each tube, was measure by an HP 8451A Diode ARRAY spectrophotometer at 532 nm. At the same time, a model system was processed as well in the same manner but without "paté".

4) Statistical analysis: the ANOVA for a five ways crossed factorial design ("paté": 0-1; sulfanilamide: 0-1; phosphoric acid: 0-1; time: 35-900 minutes; TCA: 10-15-20 %) with BMDP 2V ver. rel. 9.0 was performed.

RESULTS and DISCUSSION

Figure 1 shows the calibration curves for the MA-TBA complex developed at 35 minutes in a thermostatically controlled water-bath at 100 °C, and 15 hours in the dark at room temperature. The two curves have the same x-intercept, but different slopes, which indicates that the absorbances are different during the studied incubation times. This effect can be observed in Figure 2, which shows the absorption spectras of the MA-TBA complex for 35 and 900 minutes. It could be due to the heating effect which increases the reaction velocity between TBA and MA.

A standard containing 1x10E-8 moles of TMP/5ml resulted in an absorbance of 0,147. This absorbance value is the same as the value reported by Witte (1970), the only difference is that the former value was obtained with an incubation time of 35 minutes in boiling water, whereas Witte's value was with 900 minutes at room temperature. In this work the same standard incubated during 900 minutes gave an absorbance of 0,05135. In accordance with these results, the absorbances obtained with different times are not comparable. Thus, there is less sensitivity to the method at 900 than 35 minutes of incubation time. At the same time, the recovery percentages in samples with "paté" were less during 900 than 35 minutes. The best recovery was

obtained for 10 % of TCA solution and without SA solution: 90.97 %. This value is similar to those obtained by other authors for an extraction TBA method (Sinnhuber, 1958; Witte, 1970). The K value for extraction, which was calculated from standard curves (35 minutes at 100 °C in a thermostatically controlled water-bath) and a known dilution was 1.34, which is similar to those obtained by Tarladgis (1960) for Turner's method (1954).

Table 1 shows the results of a five ways crossed factorial design ANOVA. The absorbances values were used to make this statistical analysis because the low recovery percentages for 900 minutes test caused high K values. Despite the absorbances between the two studied times were different, the ANOVA was made to study the other factors' behaviour. The significant statistical differences between "paté" and the model system are due to the MA contribution from the naturally oxidative rancidity of "paté". There are significant statistical differences with the use of SA solution, which is observed in the ANOVA results table. In the spectras of figure 3, a low absorbance peak at 532 nm can be observed when SA solution is used. This corresponds to with the Shahidi studies (1985 and 1989), in which when residual nitrite level is less than 100 ppm the sulfanilamide can also react with MA and the test underestimates the TBA values (The studied "paté" had 55 ppm of nitrite). Moreover, the ANOVA study shows a significant effect when phosphoric acid is incorporated to the extracting solution, which is in accordance with Turner (1954), but this is not observed in the recovery percentages. The significant statistical differences obtained for time in the ANOVA are, probably, due to the temperature effect upon MA-TBA complex formation, as aforementioned previously.

CONCLUSIONS

The interaction time/temperature for malonaldehide-thiobarbituric acid reaction affects the absorbance values and thiobarbituric acid test sensitivity. The 35 minutes incubation time was the best condition for malonaldehide determination. The recovery percentages were lower during 900 than 35 minutes and its lower sensitivity makes this time/temperature be rejected. The 10 % TCA solution showed the best recovery percentages for "paté", which made it preferable to other studied extracting solutions. The extractive TBA test was quick and easy to use in poorly equiped meat laboratories, especialy when a large number of samples may to be analyzed in a short period of time.

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